Genetic Engineering in Cotton

Genetic engineering of cotton is less than two decades old and is talked about in every cotton-producing country of the world. The need to induce non-cotton genes into the cotton genome came as a result of the desire to find less expensive and environmentally safe methods to control insects. When insecticides came to be known and developed, they were quickly adopted in many countries. Many producing countries started subsidy programs to promote insecticide use at a faster rate. The two main reasons for the fast adoption of insecticides and the involvement of governments are effectiveness and cost. Insecticides were more effective than other means to control insects and the benefit-cost ratio is exceptionally high. In an effort to control insects effectively and economically, the aftereffects of injurious chemicals were forgotten. But soon many countries started realizing the consequences in damage to the environment, resistance development, change in the pest complex and a continuous increase in the number of sprays. Thus, researchers started exploring the means to get away from insecticides without sacrificing effectiveness and low cost. Transgenic cotton was found to be one such option.

Development of Genetically Engineered Plants

Chromosomes carry desirable as well as undesirable genes, and conventional breeding has the limitation of transferring the whole set of chromosomes at the same time. When two parents are crossed, depending upon the mode of inheritance, transfer of a particular character to the F_1 generation can be determined. If the character is controlled by a single dominant gene, it is certain that the character will be expressed to the F_1 generation and the F_2 generation will segregate into 3:1; 1 homozygous for the character, 2 heterozygous for the character and 1 homozygous for non-existent of the character. Though the objective for the target gene has been achieved in the first case, there is no mechanism to recover all other genes of the recipient parent. Genetic engineering provides a mechanism to add or delete a single gene to and from a genotype.

Biotechnology can be defined as the use of biological organisms or processes in any technological application. Genetic engineering is a division of biotechnology related to altering the properties of biological organisms. Genetic engineering alterations can be made at a single gene level. However, development of a transgenic genotype involves identification of a suitable gene capable of producing a particular characteristic in the plant, isolation of such a gene and induction into the target species. The three-stage developments have moved very fast during the 1980s. According to Jenkins (1990), Agracetus developed the first transgeniccotton plants reaching the field testing stage. However, the transgenic plants that effectively killed the bollworm in the laboratory failed to exhibit its effectiveness under field conditions (Jividen 1996). Just a year later Monsanto came up with another Bt gene that was capable of controlling bollworms under field conditions. Now Monsanto, the largest biotech company in the world, also owns Agracetus. Recently, the company entered the cotton seed business, particularly with the purchase of Stoneville Pedigree Company. Currently, Monsanto is offering Stoneville Pedigree Company for sale and is in the process of acquiring Delta and Pine Land Company based in Mississippi. Delta and Pine Land Company is the largest cotton seed company in the world.

Following are important stages in the development of transgenic cotton in the world:

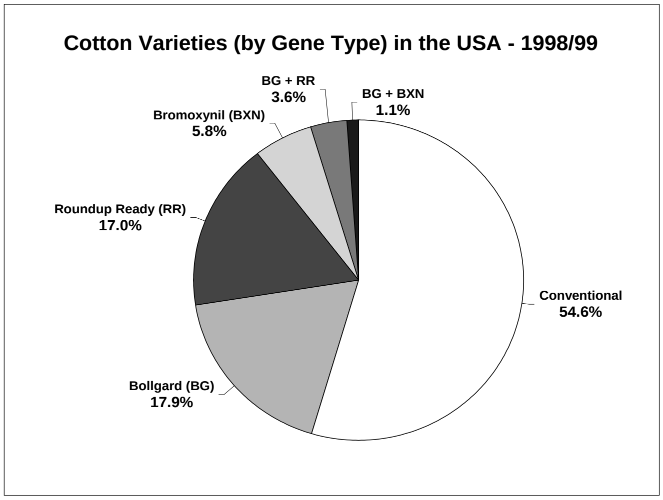
1980	Work on transgenic cotton started
1983	First transgenic cotton plant was developed
1985	First transgenic plant was transferred to the commercial side
1986	First field testing of Bt cotton
1993	First commercial demonstration trials (BXN)
1993	First regulatory approval for commercial production
1995	First commercial cultivation of herbicide tolerant transgenic cotton
1995	Seed production for commercial adoption of Bt cotton
1996	First commercial cultivation of Bt cotton
1997	First commercial cultivation of stacked gene varieties, insect plus herbicide resistant varieties

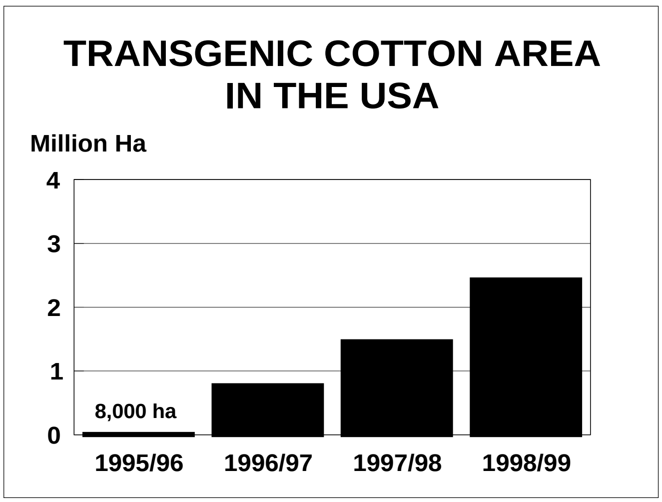
Area under Transgenic Cotton

In the early 1990s, the ill effects of insecticide use became more evident. Farmers who for many years had been hearing about Bt cotton became impatient and wanted to have the technology availed as early as possible. In this effort, some of the regulatory processing and field-testing were hastily completed. However, 1996/97 was the first time in history when Bt cotton was cultivated on a commercial scale in the USA and Australia. Bt cotton has been adopted faster than expected. It is estimated that in 1998/99, 45% of the total cotton area in the USA was planted to transgenic varieties.

The Bt gene derived from the soil bacterium *Bacillus thuringiensis* is not equally effective against all bollworms. The toxin produced by the gene Cry1A is most effective against the tobacco budworm *Heliothis virescens*. However, the gene is also quite effective against the cotton bollworm *Helicoverpa armigera* and other bollworms, but is almost non-effective against sucking insects. Thus, Bt cotton is not suitable for cultivation in all countries and in all areas. In the USA, Bt cotton is most successful in the Southeast, particularly Alabama and the Delta region, where the cotton bollworm is a major pest.

Outside the U.S., Bt cotton was commercially cultivated in Australia in 1997/98. During 1998/99, Bt cotton was commer-





Transgenic Cotton Area 1998/99

Country	Hectares
Argentina	8,000
Australia	80,000
China (Mainland)	52,000
Mexico	40,000
South Africa	12,000

cially grown in Argentina, Australia, China (Mainland), Mexico and South Africa. These countries have not yet developed their own commercial varieties carrying Bt genes and they have to rely on either Monsanto or Delta and Pine Land Company to provide transgenic varieties.

It has been confirmed in the USA, after many years of research, that the Bt gene in cotton has no deleterious effects on the plant. It does not have any adaptability problems either. But, before a variety is brought into commercial production in any country, it requires experimentation of that variety under local conditions. Trials on transgenic varieties have been completed in Spain, which are ready to go for commercial production. If a transgenic variety is cultivated in Spain, the variety approval procedure permits it to be cultivated in Greece also. Thus Spain and Greece may be the next countries adopting Bt cotton. Trials are almost near completion in Zimbabwe, which may also be planting Bt cotton in 1999/00. Trials are proceeding in Colombia, Bolivia, Brazil, El Salvador, Greece, India, Israel, Paraguay and Thailand.

Technology Fee

Transgenic cotton is not available for free. Commercial production of Bt and herbicide tolerant varieties is conditional on a fee to be paid to owners of these genes. The ability of the cotton plant to tolerate herbicides over the top of the plant and the plant's ability to produce a specific toxin are heritable characters. Once such resistant genes are inducted into the cotton plant they are automatically transmitted to the next generation. Farmers can keep the seeds and use them year after year. In the USA and Australia, this has been avoided through agreements signed with farmers that prohibit the storing of seeds for next year and also the transfer of seeds to other growers.

Agreements with biotech companies may or may not have been abused and illegal transfer of seed may or may not have taken place but there is a potential threat that it might happen, which could affect the recuperation of research and development costs by biotech companies. Chances are that the technology could be leaked to other farmers and countries without payments by growers receiving the advantages of these traits. After almost five years of research, the USDA and Delta and Pine Land Company have developed a technology called "Technology Protection System (TPS) which produces infertile seeds." TPS is a clever three-gene system that forces plants to produce a toxin that is fatal to their own seeds, compelling farmers to buy new seeds every year. A detailed note on the system was published in the March 1999 issue of *THE ICAC RECORDER*.

The TPS varieties are still a few years away but there has been a lot of criticism about the impact of the self-seed sterility system. It is perceived as exploitation of poor farmers, particularly in small farming communities. Monsanto is inclined to revisit the issue before the commercial release of TPS varieties.

The technology fee is higher in Australia because farmers will save more on insecticides. In 1997/98 a Value Guarantee Program was introduced, whereby farmers would be compensated if they spent more on Bt cotton compared to conventional insecticide spraying. The Value Guarantee Program ensured that if the cost of the technology fee minus thirty Australian dollars

Technology Fee for Transgenic Cotton in the USA

Transgenic Cotton	1996 US$/ha	1997 US$/ha	1998 US$/ha
Bt cotton	80	80	80
BXN cotton	-	15	Free
Roundup Ready	-	12 = Stripper varieties 20 = Picker varieties	17 = Stripper varieties 22 = Picker varieties
Bt + Roundup Ready	-	101	101

Bt Cotton Area and Technology Fee in Australia

Year	Area Limit (%)	Technology Fee US$/ha	Area Hectare
1996/97	10	147	38,000
1997/98	15	131	64,000
1998/99	20	112	80,000

were more than the cost of conventional spraying, Monsanto would compensate for the additional expenditure.

Concerns to the Industry

In the last three years some issues related to the effectiveness, impact of non-cotton genes, etc., have come up for the consideration of owners of the technology and of public researchers. Two of these issues which have become most important—malformation/shedding of fruit in Roundup Ready cotton and development of resistance to Bt toxin—are discussed here.

Since the cultivation of herbicide tolerant transgenic cotton started in 1995, a number of farmers in the USA reported problems with the Roundup Ready varieties in 1997/98. The complaint was localized to Mississippi and so far restricted to only one season. The common observation was that the Roundup Ready transgenic cotton showed abnormal boll shedding and in some cases bolls were deformed or partially filled. Monsanto critically analyzed the issue and concluded that there is no problem with the technology and the ability of the non-cotton gene to produce a specific enzyme for interference in the pathway of amino acid production. Extremely abnormal low temperatures during May and June and spraying of herbicide even after the four leaf stage were determined to be responsible for the excessive shedding. Since then, the company has advised farmers not to spray Roundup Ready on the cotton plant after the four-leaf stage. The company's conclusions have been supported by no abnormal effects during 1998/99.

Along with the introduction of Bt cotton for commercial scale production, fears spread that insects could soon develop resistance to the Bt toxin. Such observations have sound technical reasons. Once a Bt gene is inducted into the cotton plant, the toxin is produced throughout the plant's life regardless of whether bollworms attack. All generations of a particular bollworm will feed on the plant and could quickly become immune to the toxin, just as insecticides that are sprayed only a few times during the fruiting period. And if the toxin is present in the plant all the time, insects have a higher chance of developing resistance quickly. Developers of the insect resistant transgenic cottons admitted the fact and recommended planting of refuges. Farmers were given two options:

- For every 100 hectares of Bt cotton, 25 hectares of conventional varieties (not containing the Bollgard gene) should be planted. The non-Bt variety area would be treated with any insecticide except foliar Bt products.
- Planting of 4% of the total Bt area to non-Bt varieties. No bollworm control insecticides should be used on this area. Monsanto would provide a list of prohibited insecticides but sucking insects could be controlled with any insecticide.

Either recommendation could be adopted, which would help to delay the development of resistance. Growers could use both options to allow maximum flexibility. The objective was that the susceptible population would cross with the population feeding on varieties having the Bollgard gene and consequently a hybrid population would be produced, prolonging the development of a pure resistant population. The approach seems to be working as there are no reports of resistance development yet.

Bt Cotton and Organic Production

Organic production requires the elimination of conventional synthetic pesticides and fertilizers. Under high insect pressure, the elimination of insecticides results in extremely low yields. Thus, some areas having high insect pressure, particularly bollworms, may be unfit for organic production. It was hoped that Bt cotton varieties would become an integral component of organic production and help to promote organic production. But environmentalists have been able to convince authorities in Europe and the USA that it is not environmentally safe to grow genetically engineered varieties. Consequently, organic cotton is not considered environment friendly. According to the U.S. Environmental Protection Agency, it has been officially decided that Bt cotton cannot be certified as an organic cotton even if it were grown under organic conditions.

As the system works, the currently available Bt gene, called Bollgard in the USA and Ingard in Australia, produces a specific toxin injurious to lepidopteran insects. But the mechanism of herbicide tolerance is different from toxin production. The mode of action of glyphosate lies in the inhibition of a particular enzyme synthase, which is a key catalyst in the production of aromatic amino acids. Animals do not produce aromatic amino acids and are not affected by glyphosate. According to Stewart (1991), resistance to glyphosate has been achieved by two pathways, over production of an enzyme and enhanced insensitivity of the enzyme to the herbicide. Calgene, Inc. has developed lines of cotton resistant to glyphosate from a gene extracted from the soil bacteria *Klebsiella ozeanae*. The gene codes for the enzyme, which removes the nitrile atom from the compound and thereby detoxifies it. The mechanism of resistance to Roundup Ready does not involve production of any toxin either.

Resistance to insects through production of toxin within the plant body is one aspect of genetic engineering. There could be many more applications of genetic engineering that may not require toxin production and may be even simpler than herbicide resistance. Such genotypes may include colored cotton or the improvement of a specific fiber character. These transgenic genotypes could be perfectly fit for organic production but now they will also be prohibited in organic production. Bt cotton has been debarred from organic production but still would be an important component of integrated pest management programs.

Yield Improvement

Considerable work is proceeding on various aspects of the cotton plant but none is continuing on the improvement of yield, though yield improvement has been and still is the primary plant character to be improved. The need to improve the genetic ability of the plant to give higher harvestable yield increases with the current stagnation in yields. In most countries, the agronomic management of the plant, including pest control and optimum utilization of other inputs, irrigation water and fertilizers has reached near optimization. On the other hand, cotton genetics has reached a plateau in terms of producing new varieties with higher yield potential. Breeders have made substantial contributions in order to devise a plant most suitable to growing conditions and thus avail the maximum genetic ability of the plant. There are limitations to realizing the maximum ability of the plant and those limitations vary in nature according to growing conditions. Given the limitations in various countries, high yielding as well as low yielding, a significant improvement in yield seen in the last 4-5 decades is not expected. Though expensive but alternate methods of pest control are available in the form of insecticides, these are also being researched further with heavy investments to make them more target specific, less damaging to the environment, etc. Yet, most of the genetic engineering at this time is focused on improving agronomic characteristics of plants. End-use level products are not yet available but are expected to be available soon. There is no work on yield improvement even in the offing.

Why is no work being done on yield improvement? Tomorrow's plan may include boosting the genetic ability for bearing higher number of bolls but there is no such indication at this stage. The following factors may be responsible for the lack of any work on yield improvement.

1. Yield is a quantitatively controlled multigenic character and it is difficult to work with such a complex character.
2. The three most important components of yield are boll number, boll weight and ginning outturn. The number of bolls and boll weight, the primary characters to measure the genetic ability for yield, are influenced so much by growing conditions that genetics seems to be playing a secondary role. Even if the means are developed to boost productivity, the influence of growing conditions cannot be eliminated and certainty cannot be achieved, unlike toxin production in the plant.
3. The inheritance of yield is not properly understood, which handicaps biotechnologists to initiate work on yield improvement.
4. The morphological behavior of the cotton plant results in the production of many flower buds of which only a small part is realized as productive yield. The potential for higher yields exists. Although there may be no need for increasing the upper limit, there is a need to increase the lower limit.

What is Next?

Currently, only three types of transgenic cottons are available for commercial production: Bt resistant to lepidopteran insects, cotton resistant to glyphosate (BXN) herbicide and cotton resistant to Roundup Ready (RR) herbicide. Combinations of these became available for commercial production in 1998/99. Genetic engineering has provided an opportunity to move toward directed breeding. What genetic engineering can contribute to the development of cotton in the world is probably beyond imagination at this stage. In the future, breeders and biotechnologists might be able to construct genotypes of their own choice. There will be a choice to pick and choose genes for meeting specific needs. Conventional breeding will not be eliminated but its role will change.

Not only are insecticide costs high but the availability of the appropriate gene and its expression was also a reason that insect-resistant transgenic cotton was developed first. But now, work is going on in various aspects of the cotton plant for very specific objectives. The process of inducing foreign DNA into the cotton plant has become more successful. Though there are many ways to induct a new gene into cotton, agrobacterium-mediated transformation and direct delivery of DNA with a gene gun have been commonly used in various countries. Some varieties like Coker 312 from the U.S. and Siokra from Australia can be easily regenerated by agrobacterium mediated transformation compared to others. Researchers have to transfer genes via these two varieties. But recent developments in the gene gun method have made it more popular and successful. Now hand-held gene guns are also available, which can be carried to the field to shoot grown up plants with new DNA material.

According to Monsanto, three development stages in cotton are

Agronomic stage – Like insect resistance and herbicide tolerance, other agronomic features of the plant could be improved to include resistance to a variety of other insects and pyramid resistance to any one insect.

End-use product stage – The plant system will be manipulated to produce a specific product with a higher value like very strong or long fibers, etc. Cotton in natural blue and other colors may be available soon. Reports also indicate that it is possible to produce cotton with polyester characteristics.

Biofactory stage – Cotton is grown now only for fiber production, and the bulk of the material in cotton does not have an economical use. Cotton sticks are incorporated back into the soil or used as a fuel. Some other non-traditional uses have been explored but are still not used on a commercial scale. Farmers are not paid for 60-80% of their harvest from a cotton field but only for lint. The cotton plant can be manipulated to produce specific chemicals for medicinal purposes and the crop value can be enhanced. No reports are available on the biofactory nature of the plant utility but some work is going on in this direction.

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